Expression of neurogenic markers in Alzheimer's disease: A systematic review and meta-transcriptional analysis

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Abstract

Alzheimer's disease (AD) is the most common form of dementia characterized by substantial neuronal loss and progressive brain atrophy. Animal studies have suggested that the process of adult neurogenesis might be altered at the earliest phases of disease onset. The relationship between AD progression and adult neurogenesis in the human brain is, however, not well understood. Here, we present a systematic review of the post-mortem studies that investigated changes in human adult neurogenesis in the AD brain. We present findings from 11 post-mortem studies that were identified by a systematic search within the literature, focusing on what markers of neurogenesis were used, which stages of AD were investigated, and whether the studies had any confounding information that could potentially hinder clear interpretation of the presented data. In addition, we also review studies that examined transcriptomic changes in human AD post-mortem brains and reveal upregulated expression of neural progenitor and proliferation markers and downregulated expression of later neurogenic markers in AD. Taken together, the existing literature seem to suggest that the overall level of human adult neurogenesis is reduced during the later stages of AD, potentially due to failed maturation and integration of new-born neurons. Further investigations using complementary methods such as *in vitro* disease modelling will be helpful to understand the exact molecular mechanisms underlying such pattern of change and to determine whether neurogenesis can be an effective therapeutic target for early intervention.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia and no disease-modifying therapies are currently available. Extracellular amyloid plaques and intracellular neurofibrillary tangles are neuropathological hallmarks of AD. The degree of pathology can be measured by following Braak stages, which traces the progression of neurofibrillary tangles from the transentorhinal regions (Braak I-II), to the limbic regions including the hippocampal formation (Braak III-IV), and then to the neocortical regions (Braak V-VI) (Braak and Braak, 1991; Braak et al., 1993). These stages further relate to the emergence of atrophy in the hippocampal formation, which is one of the most salient and earliest features of AD, and they also correlate with the progression of neuronal loss observed in the respective areas as well as memory loss (Bancher et al., 1993).

Since adult neurogenesis is implicated in normal functionality of hippocampal circuits (Marin-Burgin and Schinder, 2012), impaired neurogenesis may detrimentally affect the survival of adult-born neurons and contribute to defects in learning and memory observed in AD by reducing brain plasticity (Gadadhar et al., 2011). Furthermore, rodent studies have shown that several molecular factors important for AD pathogenesis, such as amyloid precursor protein (APP), presenilin 1 (PSEN), and apolipoprotein E (APOE), also play a key role in modulating adult hippocampal neurogenesis, suggesting that dysregulation of neurogenesis might be an important aspect of AD progression (Mu and Gage, 2011).

Adult mammalian endogenous neurogenesis occurs predominantly in the hippocampal dentate gyrus (DG) and in the subventricular zone (SVZ) situated throughout the lateral ventricle walls.

Type I neural stem cells originating in the subgranular zone (SGZ) in the DG give rise to type IIa/IIb neural progenitor cells and type III neuroblasts which eventually integrate in the neural network forming the granular cell layer (Fig. 1). Functionally, neurogenesis in the DG has been shown to be crucial for hippocampal dependent learning and memory including conjunctive encoding, pattern separation, pattern completion, and spatial navigation in rodent models (Lazarov and Hollands, 2016).

Neurogenic remodeling of hippocampal circuitry is also thought to play an important role in both memory persistence and transience in rodents (Richards and Frankland, 2017).

In the SVZ, type A cells are present in a layer found immediately beneath the ependymal cell layer (ECL) and represent the migrating neuroblasts. Type B cells are GFAP positive and represent the primary progenitors while type C are known as the transient amplifying cells. In rodents, the neural progenitors originating at the SVZ follow the rostral migratory stream (RMS) into the olfactory bulb (OB) (Fig. 1) where they develop into interneurons of granule cells or periglomerular cells (Lazarov and Hollands, 2016). Studies have suggested that in humans, SVZ-derived neuroblasts might be migrating to the adjacent striatum, also a region of importance for cognitive flexibility (Bergmann et al., 2015).

Endogenous neurogenesis has been generally shown to persist throughout adulthood, and its levels can drop significantly with normal aging in various mammals including rodents (Knoth et al., 2010; Kuhn et al., 1996) and non-human primates (Leuner et al., 2007), correlating with age-related cognitive decline and memory loss.

Expanding this knowledge onto humans, several studies have shown that postnatal neurogenesis also persists in the adult human brain throughout life, primarily in the hippocampus (Eriksson et al., 1998; Spalding et al., 2013). However, recent findings from postmortem human brain tissues have reported contradicting findings, where one study suggested that human hippocampal neurogenesis declines sharply to undetectable levels in adults after the first year of life (Sorrells et al., 2018), and the other study reported that endogenous neurogenesis does continue throughout aging with no significant changes in the pools of progenitor cells and immature neurons (Boldrini et al., 2018). Although several lines of evidence generally seem to support the prevailing view on the existence of human adult neurogenesis, the discrepancy of these findings point to the limitations in our current understanding of human hippocampal neurogenesis and suggest room for improvement (Kempermann et al., 2018; Lee and Thuret, 2018).

Most of our current understanding of *in vivo* adult neurogenesis has been derived from non-human animal models which helped us to identify cellular markers that can be used to label different types

of cells at each stage of neurogenesis. In postnatal hippocampal neurogenesis, for example, these markers include glial fibrillary acidic protein (GFAP) and Nestin (type I neural stem cells), doublecortin (DCX) and polysialic acid neural cell adhesion molecule (PSA-NCAM) (type IIa/IIb neural progenitor cells and type III neuroblasts), and prospero homeobox 1 (PROX1) (immature and mature dentate gyrus granule cells). The expression patterns of these markers are known to be well conserved across different species and have therefore been extrapolated to represent stages of neurogenesis in human post-mortem studies. However, the use of these 'proxy' markers of neurogenesis may be caveated by several factors: cells tend to express multiple markers at a given time; markers may be sensitive to post-mortem delay; there are variations in methods of cell quantification, and lastly none of these markers have yet been validated in human samples. These limitations are highlighted and discussed in more detail within this review. In anticipation of human-verified markers of neurogenesis, we here in present a transcriptomic analysis of several human AD profiles which complements the immunostaining studies reviewed.

It is unclear whether promotion of endogenous neurogenesis may be a directly relevant treatment target *per se* in AD, since it is unlikely that a small number of newly generated neurons might have a large enough effect to repair the damage and regenerate the degenerated neurons in late-stage AD. However, it might be possible that a small pool of new neurons generated at the early stages of AD exert enough support to prevent or slow down severe cognitive decline by contributing directly to the enhancement of memory function (Deng et al., 2010). Implantation of human cortical stem cells in rodent models of stroke has been shown to induce endogenous neurogenesis and promote angiogenesis and trophic factor release (Hassani et al., 2012; Hicks et al., 2013) and it would be interesting to investigate whether promotion of endogenous neurogenesis could confer similar benefits. Furthermore, several studies have shown that physical activity, environmental enrichment and higher levels of education promote hippocampal neurogenesis as well as improve memory (Mu and Gage, 2011; Rodríguez and Verkhratsky, 2011). Such findings might suggest that maintenance of endogenous neurogenesis throughout adulthood might contribute to cognitive resilience in AD.

In this systematic review, we discuss findings from studies that have investigated changes in human adult neurogenesis in AD by collating all relevant data generated from human post-mortem studies that can be found in the existing literature. We summarize their findings primarily focusing on the neurogenesis markers that were used to measure the relative changes in the AD brain compared to controls. We also discuss the findings in relation to AD stage of progression and any possible confounding factors that might have been introduced to the studies.

2. Material and Methods

2.1 Systematic Search

Following the International Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009), we undertook a systematic search for literature indexed in Medline and Embase up to 18th of June 2018. Full search terms utilized in the individual databases are shown in supplementary material (S1).

Relevant literature reviews (von Bohlen Und Halbach, 2007, 2011) were used as guides in selecting appropriate neurogenic and proliferation markers which identify the different stages of neurogenesis. The possible aliases and search terms for these individual markers were then identified using GeneCards (www.genecards.org (Stelzer et al., 2016)), and are shown in S1 and described in Box1. Since the aim of this review is to investigate neurogenesis in AD, articles investigating the expression of proliferation markers (Ki67 and PCNA) were only included if they also investigated the expression of at least one neurogenic marker listed in Box1.

Only peer-reviewed primary research articles written in the English language were considered. Reviews and conference abstracts were excluded. Articles passed the inclusion criteria if they: 1) were carried out with post-mortem brain samples of AD 2) measured the expression of neurogenic markers listed in Box1 in the neurogenic niches of the brain (SVZ and SGZ) and 3) were compared with non-demented controls. References of resulting articles were searched for additional relevant studies.

Retained articles were evaluated against the inclusion criteria by a second independent reviewer (H.L.). Data was manually extracted from the studies and is listed in table 1. Overall results extracted from the different studies are shown in matrix form in Table 2. The terms higher (↑), lower (↓) or unchanged (↔) used in table 1 and 2 refer to the corresponding changes in expression of neurogenic or proliferation markers relative to the control group within the study, as identified by the authors of the study themselves.

Due to the variability in techniques and outcome measures in these studies, a relevant meta-analysis or other statistical summary is not reported here.

2.2 The meta-AD transcriptional profile

A total of 30 independent AD profiles were generated from published transcriptional data (Gene Expression Omnibus (GEO) database (Barrett et al., 2013)), and a meta-AD profile was generated that consists of a list of genes with the corresponding sum of up/down (counted as +/- 1) regulation calls across these profiles (Supplemental material Table S1-3, Fig.3A-C). The effects of different brain regions were controlled for by treating it as a categorical covariate when combining data from different brain regions.

In addition to looking at a panel of AD profiles, we examined a set of expression data corresponding to a range of clinical dementia ratings (CDR) (GSE84422 – 19 brain regions from 125 individuals (Wang et al., 2016) and another set of expression data corresponding to a range of Mini-Mental State Examination (MMSE) scores (GSE1297 - hippocampi from 31 individuals (Blalock et al., 2004). Here, we performed a linear mixed model for each gene probe to see which genes best explain cognitive decline while treating brain region, sex and race as covariates where given. The numbers given are the Z-scores associated with the correlation between gene expression and cognition score (Supplemental material Table S4-6, Fig.3D-F). Further, we assessed to what extent genes whose expression explained the decline in cognition were also seen to vary in expression across a panel of AD profiles.

3. Results

The database searches identified 1661 potential articles, of which 50 potentially met the inclusion criteria based on the title and abstract, but only 8 fully met all inclusion criteria. An additional 3 articles were identified from the references of these 8 studies, yielding a total of 11 articles included in the current systematic review (Fig. 2.). To be able to interpret the findings appropriately, the conclusions from the individual articles will be presented according to the neurogenic stage which the markers used identify (Fig.1.).

3.1 Stage 1 (proliferation) and stage 2 (differentiation)–GFAP, Msi-1, SOX2, Nestin

Seven articles investigated changes in stage 1 and stage 2 markers. Two studies reported an upregulated expression of the astrocytic marker GFAP, expressed in the radial-glia-like stem cells, in the DG in the severe stages of AD (Braak V-VI) (Boekhoorn et al., 2006; Ekonomou et al., 2015). GFAP expression was, however, reduced in Braak stages III-IV, suggesting reduced glial support in the earlier disease stages (Ekonomou et al., 2015).

Expression of Msi-1, a marker of undifferentiated, proliferative cells, was upregulated in the DG in Braak stage II and downregulated in Braak stage IV and VI (Perry et al., 2012). Downregulated expression of Msi-1 was also observed in the later disease stages in AD SVZ (Perry et al., 2012; Ziabreva et al., 2006); but only reached statistical significant difference in one study (Ziabreva et al., 2006). Importantly, both studies (Perry et al., 2012; Ziabreva et al., 2006) reported a correlation of Msi-1 expression with choline acetyltransferase (ChAT) immunoreactivity suggesting a link between reduced cholinergic activity and reduced progenitor number.

Expression of the neural stem cell marker SOX2 was significantly reduced in the DG in Braak stage VI in two studies (Briley et al., 2016; Crews et al., 2010) whilst another study reported no change in SOX2 expression in the AD DG (Gomez-Nicola et al., 2014). However, the disease stage investigated, or extent of pathology were not presented in the latter manuscript. Interestingly a study investigating neurogenic changes in AD and non-demented patients with Alzheimer's neuropathology (NDAN),

found that SOX2+ cells were more abundant in NDAN DG compared to AD or MCI patient DGs (Briley et al., 2016) . Consistently with the Boekhoorn study, AD patient DG exhibited reduced numbers of SOX2+ cells, suggesting a reduction in the number of neural stem cells in AD (Braak stage VI). SOX2 expression was, however, elevated in the DG of both MCI (Braak stage II-V) and NDAN patient groups (Braak stage IV-VI) when compared to controls.

Importantly, these authors found that a percentage of cells in the human DG were expressing both SOX2 and the late postmitotic marker NeuN. AD patient DG exhibited reduced numbers of SOX2+/NeuN- cells as well as SOX2+/NeuN+ cells, suggesting a reduction in the number of both neural stem cells as well as mature postmitotic neurons in AD. Conversely, MCI patients exhibited increased SOX2+/NeuN- cells and decreased SOX2+/NeuN+ cells whilst in NDAN individuals, both SOX2+/NeuN- cells as well as SOX2+/NeuN+ cells were increased when compared to controls (Briley et al., 2016). This suggests that NDAN individuals hold higher potential for not only generating more neural stem cells, but also for these cells to survive long enough to become mature neurons, whilst AD individuals have reduced numbers of stem cells as well as a reduced number of postmitotic neurons.

Expression of the neural stem/progenitor cell marker Nestin was unchanged in the AD ependymal cell layer (ECL) of the SVZ in two studies (Ekonomou et al., 2015; Ziabreva et al., 2006), and increased in higher Braak stages in AD SVZ (Perry et al., 2012; Ziabreva et al., 2006), SGL and GL (Perry et al., 2012). This increased expression might not necessarily indicate increased migration or targeting, as expression of DCX and B-tubulin remained unchanged in the subgranular and granular layers of the DG (Perry et al., 2012) . A later study found no such changes in Nestin expression in the SVZ or in the DG throughout different Braak stages (Ekonomou et al., 2015). It is unclear whether the changes observed by Perry could have been due to re-induced Nestin expression in glial cells following neurotoxicity, as has been previously reported (von Bohlen und Halbach, 2011).

3.2 Stage 3 (migration) –PSA-NCAM, DCX, TUC-4/CRMP-4

Once immature neurons commit to a neuronal lineage, they stop expressing Nestin and start expressing PSA-NCAM and Doublecortin (DCX). A total of nine articles investigated the expression of stage 3 markers.

In one study, total NCAM expression in AD brains was similar to controls in both its polysialylated form and other forms (Gillian et al., 1994). The cohort sizes used, however, were relatively small and the disease severity was not reported. Later studies found that PSA-NCAM immunoreactivity was significantly higher in AD SGL and GL (Jin et al., 2004b; Mikkonen et al., 1999; Perry et al., 2012).

In one of the first studies focused on investigating neurogenesis in AD, PSA-NCAM, DCX and TUC-4(/CRMP-4) expression were found to be increased in the hippocampi of AD when examined by western blotting, correlating with AD severity (Jin et al., 2004b). The upregulation of DCX and TUC-4 in the AD GL was also verified by immunohistochemical analysis. While this was a pioneering study in terms of the range of neurogenic markers studied in the AD brain, it did present a number of caveats: the cohort numbers were relatively small; the control cases in the western blot analysis were much younger than AD cases; and the disease severity and gender of AD cases were unknown.

Two later studies also observed an overall increased expression of DCX in the later AD stages in the DG (Ekonomou et al., 2015; Perry et al., 2012), whilst another study reported reduced expression of DCX in the DG of AD (Braak VI) compared to non-demented controls and Braak stages I-II (Crews et al., 2010). Another study found no difference in DCX expression in DG of presenile AD, and reported high variability in the numbers of DCX-positive granular elements between subjects (Boekhoorn et al., 2006). DCX is considered a marker for late mitotic neuronal precursors and early postmitotic neurons. However, it has also been reported to label astrocytes (Verwer et al., 2007) and dormant cells (Kremer et al., 2013; Marti-Mengual et al., 2013) in non-neurogenic regions. Furthermore, DCX expression is particularly sensitive to post-mortem delay (Boekhoorn et al., 2006), which is a factor that inevitably differs greatly between the five studies that investigated it (ranging between an average of 5h

(Boekhoorn et al., 2006) to 17.5h (Ekonomou et al., 2015) in the AD groups). These results therefore need to be interpreted with caution.

3.3 Stages 4 (axonal and dendritic targeting) and 5 (Synaptic integration) – Calretinin, NeuN, Calbindin, β-III-tubulin, MAP2a,b,c, HuC/D

Once the newly generated neurons become postmitotic they start transiently expressing the calcium-binding protein calretinin and the widely used neuronal marker NeuN. After approximately 2-3 weeks in this postmitotic stage, calretinin is exchanged for calbindin in mature granule cells, which functionally integrate in the hippocampus. NeuN expression is sustained in these mature cells. Six articles investigated the expression of stage 4 and 5 markers.

One study, which did not provide information regarding disease stage, reported upregulated labelling of Calretinin in the AD DG (Gomez-Nicola et al., 2014). Expression of Calbindin and NeuN was found to be unchanged in the AD hippocampal region via western blot analysis (Jin et al., 2004b) and β-III-tubulin expression was also unchanged in AD SVZ and DG in another study (Perry et al., 2012) . Expression of MAP2c was also unchanged in an additional study (Li et al., 2008), whilst expression of MAP2a,b isoforms was significantly decreased in AD DG, suggesting that it is the maturation of neurons that is being compromised (Li et al., 2008).

Expression of the neuronal marker protein HuC/D was decreased in the DG of late Braak AD stages, however, double PCNA/HuC/D immunolabelling was unchanged suggesting that there was no overall change in neural progenitors (Ekonomou et al., 2015).

3.4 Proliferation markers – Ki67, PCNA

PCNA is also expressed in non-neuronal cells and has previously been shown to be upregulated in AD glial cells with a trend towards increased expression correlating with increased AD pathology, along with Ki-67 (Wharton et al., 2005). Within the articles included in this review, two studies have

demonstrated elevated Ki-67 expression in the AD hippocampus (Boekhoorn et al., 2006; Gomez-Nicola et al., 2014) suggesting that overall proliferation might be elevated in AD.

3.5 Filtration of articles

Since the articles herein reviewed were of variable quality and in some cases exhibited contrasting results, we further filtered the search to collate the articles that included the most information we deemed relevant to the question at hand (Table 3). The filtering criteria we set to assess quality of the publication were related to 1) whether control cases were defined and age-matched to the AD cases, 2) whether a disease severity scale (Braak stage) was reported, 3) whether multiple neurogenic or proliferation markers were investigated and 4) whether any confounding pathologies were discussed. Overall, only two articles (Boekhoorn et al., 2006; Ekonomou et al., 2015) passed all four filtering criteria.

Both articles reported an upregulated expression of GFAP suggesting extensive astrogliosis in severe AD (Braak stage V-VI). The Ekonomou study, however, also reported reduced GFAP expression in Braak stages III-IV (Ekonomou et al., 2015). Since GFAP identifies both neural stem cells and astrocytes it is difficult to determine whether this Braak stage variability suggests a differential neurogenesis or gliogenesis in different stages of AD. Ekonomou reported no change in expression of the early neural precursor Nestin in AD (Ekonomou et al., 2015). The two studies report conflicting findings in relation to DCX expression with Boekhoorn being unable to draw any conclusions from the data and Ekonomou suggesting that DCX-positive cells were more common in DG of individuals with higher Braak stages (Boekhoorn et al., 2006; Ekonomou et al., 2011). However, both studies adamantly report that DCX expression was too low and variable to draw significant conclusions. Ekonomou et al., also detected a lower number of HuC/D-positive postmitotic early neurons in the DG in individuals with higher Braak stages, whilst double immunolabelling of HuC/D-PCNA was unchanged suggesting no alteration in the number of neural progenitors in AD (Ekonomou et al., 2015).

3.6 Transcriptional changes in neurogenic/proliferation markers in AD

To validate the alterations in levels of expression of neurogenic and proliferation markers in AD, we investigated differences at the transcriptional level. To this end, human AD post-mortem studies deposited in the NCBI GEO database (www.ncbi.nlm.nih.gov/geo/) were collected by querying the series repository with 'Alzheimer's' as key term and restricting the output to human brain sample data. This resulted in a total of 30 independent AD profiles generated from 11 expression series. Significance was assessed with a student's t-test on the difference between the AD and healthy subsets. Probes were assigned to genes, and the probe with the largest expression change was selected from AD subsets. The expression change values shown in supplementary tables (Table S1-6) are scaled according to $2(d-c)/(d+c)$, where *d* and *c* are the average disease and control levels (Fig.3.). Significant expression changes below 25% were taken to be biologically irrelevant and dropped from subsequent analysis.

A meta-AD profile was created by summing the up and down calls on each gene across the expression set (Table S1, Fig. 4A). This profile demonstrates that throughout the different AD studies, expression of neural progenitor markers and the cell proliferation marker, *MKI67,* were upregulated whilst expression of the later neurogenic markers was mainly downregulated. The meta-AD profile was then restricted to a set of 13 studies that investigated the hippocampus and further subdivided into studies investigating transcriptomic changes in early AD and late AD. AD severity was defined as identified by the individual studies themselves. Only eight markers appeared to be altered in the early AD stage, corresponding to a handful of studies (Table S2, Fig.4B), whilst the majority of late AD studies demonstrated upregulation of neural progenitor markers and downregulation of later neurogenic markers (Table S3, Fig. 4C).

Although there were fewer datasets that reported cognitive measure outcomes such as MMSE and CDR scores alongside transcriptomics data, we nevertheless decided to investigate to what extent gene expression changes in neurogenic markers can explain cognitive decline. Analysis of a study investigating the correlation of gene expression with MMSE scores (GEO accession GSE1297 (Blalock

et al., 2004)), identified *GFAP* and *MK167* as significant anti-correlative predictors of MMSE, where the genes are correlated with moderate cognitive decline (MMSE>15) and mild cognitive decline (MMSE>20), respectively. Expression levels of *NEUROD1, NEUROD6, CRMP1, TUBB, ELAVL4* were positively correlated with MMSE, and therefore, negatively correlated with AD. *NEUROD6* was identified as a significant predictor of moderate cognitive decline (MMSE>15) (Table S4, Fig. 4D.). An additional study (GEO accession GSE84422 (Wang et al., 2016)) was investigated as it covered a wide range of brain regions in a large cohort of individuals with a full range of Clinical Dementia Rating (CDR) scores (0-5). The expression of neural progenitor markers (*VIM, MSI1, NES*), *GFAP*, and *MKI67* were positively correlated whilst most of the later neurogenic markers (*NEUROD2, NEUROD6, CRMP1, NCAM1, DCX, TUBB, CALB1, MAP2, ELAVL2, ELAVL4, RBFOX3*) were negatively correlated with CDR (Table S5, Fig.4E). A similar result was observed in an analysis restricted to a CDR score of less than 2 (Table S6, Fig.4F). Taken together, our analysis validates significant changes in expression of neurogenic markers in the AD brain, including the hippocampal region, and suggests that these expression changes are correlated with cognitive decline.

4.Discussion

A systematic review presented in this study suggest that neurogenesis is reduced in the later stages of AD. We find that expression of neural progenitor and proliferation markers is transcriptionally upregulated whilst that of later neurogenic markers is downregulated in the hippocampus of late AD brains. Based on these findings, we propose the hypothesis that neurogenesis is reduced in late AD due to failed maturation and integration of new neurons.

Studies of adult neurogenesis in rodent models of AD have yielded contradictory findings, with some studies suggesting a reduction in proliferation (Demars et al., 2010), survival (Verret et al., 2007), and maturation (Li et al., 2009) of new-born neurons and others suggesting increased proliferation and differentiation of the same cells (Jin et al., 2004a; Lopez-Toledano and Shelanski, 2007) [for a more detailed review, see (Mu and Gage, 2011)]. Interestingly, a study investigating the different stages of neurogenesis in different stages of neurodegeneration in PS1/PS2 knockout mice, found that levels of neurogenesis in the DG are directly correlated to the severity of neuronal loss in the hippocampus. Furthermore, whilst early neurodegeneration triggered an upregulation of adult neurogenesis in this study, late-stage neurodegeneration led to the downregulation of the process, which is in line with the overall findings from this systematic review (Chen 2008).

4.1 Neuronal maturation is compromised in AD

According to the studies reviewed here, there is a distinction between the expression of neurogenic and glial markers in AD, with greater compromise in neuronal maturation. Both IHC and transcriptomic studies suggest that expression of markers/genes associated with neuronal maturation are downregulated in AD. Interestingly, a recently published study reported similar findings in a normal healthy brain (Mathews et al., 2017). The early and intermediate phases of neurogenesis were unchanged and stem cell pools remained consistent, while proliferation and number of mature neurons were reduced with aging, possibly due to alterations in the hippocampal microenvironment

which affect the expression of genes regulating maturation and migration of new neurons (Mathews et al., 2017).

4.2 Neurogenic changes in the AD SVZ are not linked to changes in the AD DG

Regional differences indicated that whilst most studies focused on neurogenic changes in the DG, only three IHC studies investigated changes in the SVZ (Ekonomou et al., 2015; Perry et al., 2012; Ziabreva et al., 2006), with the expression of the majority of neurogenic markers investigated being unchanged in AD SVZ compared to controls. Neurogenic changes in the AD SVZ are therefore still a point of debate and we suspect there is not enough information available to draw a conclusion regarding the matter. However, it appears that SVZ changes do not directly mirror changes in the DG and vice versa.

4.3 Correlation between neurogenesis and cholinergic pathology

Two of the studies reviewed exhibited a correlation between Msi-1 expression and ChAT immunoreactivity. Acetylcholine has been shown to promote the proliferation of neural stem cells and is known to mediate synaptic plasticity (Mitsushima et al., 2013) and is therefore a feasible regulator of neurogenesis. Animal studies have shown that lesions to the cholinergic basal forebrain neurons in adult rats led to reduced neurogenesis in the dentate gyrus and olfactory bulb, as well as increased apoptosis in the neurogenic regions of the rat brain, whilst systemic administration of a cholinergic agonist caused an increase in proliferation and short-term survival of neuronal progenitors in the rat DG (Mohapel et al., 2005). Importantly, an additional study observed ectopic expression of Msi-1 in a significant number of neurons containing cytoplasmic inclusions in severe AD, suggesting an involvement of the protein in the pathogenesis of the disease (Lovell and Markesbery, 2005). This finding could suggest that the observed correlation of Msi-1 expression and cholinergic pathology in AD neurogenic regions might not be a direct reflection of alterations in neurogenesis *per se* and such results should therefore be interpreted with caution.

4.4 Correlation between neurogenesis and vascular pathology

In their study, Ekonomou et al., excluded any cases presenting with cerebrovascular pathology from their cohort (Ekonomou et al., 2015), whilst Boekhoorn et al., discussed any vascular associated changes within their findings (Boekhoorn et al., 2006). They found that Ki67 expression was elevated in AD specifically in areas of vascular pathology, however they specify that these proliferative changes are most likely to be representing aberrant and non-functional Ki67 expression and are therefore not defining neurogenesis (Boekhoorn et al., 2006). Several other studies have shown that neural progenitors are indeed increased in areas of cerebrovascular pathology in cases of Vascular dementia, cerebral small vessel disease, stroke, ischemic injury and cerebral infarction (Ekonomou et al., 2011; Ekonomou et al., 2012; Jin et al., 2006; Macas et al., 2006; Minger et al., 2007). Furthermore, neural progenitor cells in the SVZ migrate to the area of cerebrovascular injury (Arvidsson et al., 2002; Ekonomou et al., 2011) and are capable of differentiating into immature neurons (Ekonomou et al., 2011). The remaining articles discussed in this review do not mention cerebrovascular pathologies in their cohorts. The factors regulating this mechanism are currently unknown, but such findings do suggest that vascular changes must be analysed when investigating neurogenic changes in AD.

4.5 Differing methodologies, medications and changes in gliogenesis might contribute to variability observed in the studies

The studies selected for this review exhibit some conflicting results which are difficult to interpret. Such variability in these studies might be related to cases exhibiting concurrent pathologies (such as vascular changes or Lewy body pathology), to only one antibody being used as a marker of endogenous neurogenesis, therefore only representing one stage of the process, and to numerous articles not relating their findings to disease severity (such as Braak staging). We chose to review articles in which IHC was the main technique utilized to look at changes in neurogenesis. Studies that do not take into account regional staining (as in western blot analysis (Jin et al., 2004b)) risk diluting out potential differences between AD and control tissues, whilst IHC takes into account heterogeneity in the brain and provides a region-specific outcome. Technical differences still contribute to the results

obtained between studies especially if different fixation times, antibodies and antigen retrieval methods are used. Moreover, the studies reviewed used different methods of quantifying immunoreactivity in their samples with some quantifying optical density or percentage area of immunoreactivity and others providing an actual cell count of immunopositive cells.

None of the identified studies reported on the use of specific medications in controls or Alzheimer's patients. Unreported medication use, especially if differing between controls and AD subjects, could be a potential confounding factor. Antidepressants are commonly prescribed to dementia patients and several studies have shown that treatment with antidepressants leads to improved neurogenesis (Boldrini et al., 2012; Boldrini et al., 2009; Gatt et al., 2018) including a recent study showing that SSRI treatment in DLB/PDD elevated DCX expression which, in turn, correlated with preserved cognition (Gatt et al., 2018). Similarly, alterations in neuroinflammation are related to neurogenesis (Fuster-Matanzo et al., 2013) and nonsteroidal anti-inflammatory drug treatment has been shown to reduce the number of activated glial cells (Heneka et al., 2005; Mackenzie and Munoz, 1998). The role of astrocytes and microglia in modulating the neuroinflammatory response in AD has also been well investigated (Hopperton et al., 2018; Phillips et al., 2014). GFAP-positive glial cells play an important role in neurogenesis providing metabolic support, regulating synaptic formation and transmission, releasing trophic factors as well as playing an important role in neuroinflammation. Studies reviewed here have shown that GFAP immunoreactivity was increased in the more severe disease stages in AD. It is difficult to interpret whether GFAP expression elevation is due to increased gliogenesis or increased number of glia-like stem cells. It would be interesting to determine how GFAP expression relates to underlying pathological burden, and whether there is a therapeutic window in which microglial or astrocyte stimulation could be used to promote endogenous neurogenesis.

Further, whilst Msi-1 and SOX2 expression appears to be downregulated in the IHC studies reviewed, transcriptional analyses revealed an upregulated expression of these genes in AD profiles. Contrastingly, DCX, Tuc-4 and NCAM expression was upregulated in IHC studies and downregulated in

AD transcriptional studies. It is difficult to interpret what could be causing these differences, however, as described above, there are numerous factors that question the use of Msi-1 and DCX as markers of neurogenesis in IHC which could partially explain the diverging findings.

Overall, it is still unclear how underlying AD pathology affects the generation of new neurons in the adult brain, however, since a multitude of factors affect this process including neuroinflammation, vascular pathology and treatment with antidepressants, it is imperative for such confounding factors to be considered when investigating neurogenesis in AD postmortem tissue.

4.6 Limitations and recommendations

This review has important limitations. Using post-mortem tissue to investigate the expression of neurogenic markers is limited to the specific disease stage at which autopsy took place. Multiple labelling is therefore crucial to distinguish whether an increase in neural progenitors signifies an increase in mature neurons (and therefore neurogenesis) or whether these progenitors fail to mature to functional neurons. Furthermore, since several markers, such as DCX, are expressed by various cell types at multiple neurogenic stages, one must take care in comparing studies which focus on only one marker. The markers we have outlined as associated with neurogenesis were mainly identified as such, when studying neurogenesis in animal models. This is not an ideal scenario as several discrepancies exist in the expression of neurogenic markers in rodents compared to humans and extrapolating rodent findings to human studies has clear limitations. As Briley et al., observed in their study, SOX2 expression overlaps with that of the neuronal marker NeuN in the human but not in the murine DG, where SOX2 expression is restricted to undifferentiated NSCs (Briley et al., 2016). Furthermore, it has been shown that animal neural stem cells can differ significantly from the human equivalent such as having different paces of maturation as demonstrated by Otani and colleagues (Otani et al., 2016), which leads to the possibility of different set of markers being expressed at different stages of neurogenesis, or even the same markers serving different functions at each stage. Taken together, these observations highlight that the field needs to establish a library of human-specific neurogenic

markers that are robust and clearly interpretable. One could perhaps start by trying to identify which markers are human-relevant through *in vitro* modelling of human neurogenesis stages from neural stem cells to mature functional neurons. For example, a time-course characterisation of human embryonic or induced pluripotent stem cells that are differentiating into hippocampal neurons could be done by analysing the whole transcriptome at a single-cell level. Given that several protocols for generating dentate gyrus granule cells and hippocampal pyramidal cells are already available in the literature (Sakaguchi et al., 2015; Sarkar et al., 2018; Yu et al., 2014), a single-cell analysis on the cell-types generated during the differentiation process could be readily achieved. Such characterisation approach would not only help us to validate cell-type and stage-specific markers of human neurogenesis but also to develop an accurate understanding of how neurogenesis is affected in the disease context.

The transcriptional profiles investigated in this review were not generated from single-cell transcriptomic analysis but rather from brain tissue comprising multiple cell types. It is therefore difficult to assess whether the transcriptional changes observed are directly attributable to those cells undergoing adult neurogenesis in the DG.

However, animal models of neurogenesis have previously shown that transcriptional changes in neurogenic markers correlate well with changes in endogenous neurogenesis (Burger et al., 2008; Inoue et al., 2015; Juliandi et al., 2015). Our analyses of human transcriptomic datasets show that in the hippocampus, several neurogenic marker genes are differentially expressed between AD and healthy patients, and the expression pattern of these data identified by meta-profile analysis are in line with that of IHC experiments reviewed in this study. Overall, both IHC and transcriptomic analyses suggest that whilst the expression of proliferation marker is increased, late neurogenic markers are downregulated in the hippocampus in AD.

We suggest that the identification of human-relevant markers of neurogenesis as well as investigations into transcriptomic changes and temporal expression of neurogenic markers at the

single-cell level would be imperative to better elucidate the changes occurring in the key player cell-types of adult neurogenesis in AD.

5. Conclusions

Taken together, the findings reviewed herein seem to suggest that numbers of neural progenitors are increased or unaltered in AD compared to controls. However, maturation of these progenitors into new neurons is compromised in late AD (Boekhoorn et al., 2006; Briley et al., 2016; Ekonomou et al., 2015; Jin et al., 2004b; Li et al., 2008). This model also seems applicable at the transcriptional level (Fig. 4). Since most post-mortem human studies reviewed herein were carried out in the later stages of the disease (Braak V-VI), it is not possible to draw any conclusion with regards to alterations of neurogenesis at the early stages of AD. However, we can conclude that overall, the generation of fully functional new neurons is reduced in late Alzheimer's disease.

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Table and figure legends

Table 1.

Main findings of 13 studies on expression of neurogenic markers in post-mortem human neurodegenerative disease brain samples. The terms higher (↑), lower (↓) or unchanged (↔) used in the table refer to the corresponding changes in expression of neurogenic or proliferation markers relative to the control group within the study, as identified by the authors of the study themselves. AD: Alzheimer's disease, NDAN: non-demented patients with Alzheimer's neuropathology, CJD: Creutzfeldt-Jakob disease, PMD: postmortem delay, MMSE: mini mental state examination, SGZ: subgranular zone, ECL: ependymal cell layer, DG: dentate gyrus, WB: western blotting, IHC: immunohistochemistry, GCL: granule cell layer, SVZ: subventricular zone, ICC: immunocytochemistry, CERAD: Consortium to Establish a Registry for Alzheimer's Disease, APOE: Apolipoprotein E, CA: Cornu Ammonis.

Table 2.

A summary of the expression changes observed in AD compared to controls in the 13 different studies reviewed. The terms higher (↑), lower (↓) or unchanged (↔) used in the table refer to the corresponding changes in expression of neurogenic or proliferation markers relative to the control group within the study, as identified by the authors of the study themselves. SGZ: subgranular zone, ECL: ependymal cell layer, DG: dentate gyrus, WB: western blotting, IHC: immunohistochemistry, GCL: granule cell layer, EC: entorhinal cortex, SVZ: subventricular zone, NDAN: non-demented patients with Alzheimer's neuropathology, AD: Alzheimer's disease.

Table 3.

Filtering criteria for the 13 Alzheimer's disease (AD) related articles yielded from the systematic search.

Box 1.

List of neurogenesis and proliferation markers discussed within this review.

Fig. 1. Diagrams showing the neurogenesis process in the dentate gyrus (DG) and the subventricular zone (SVZ) of the adult mammalian brain. The markers listed are those known to mark the corresponding five stages of the neuronal maturation process (primarily from rodent studies). RMS: rostral migratory stream, OB: olfactory bulb.

Fig.2. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et al., 2009) flow diagram for systematic search and identification of studies meeting inclusion criteria for systematic review. Inclusion criteria were as follows: 1) studies evaluating post-mortem brain samples of a neurodegenerative disease, 2) studies measuring neurogenic markers and 3) studies including patient cases and non-demented controls. Exclusion criteria were as follows: 1) studies not in English; 2) non-primary literature; 3) animal research; 4) non-immunohistochemical or western blotting analysis as primary outcome variables; 5) case studies without controls.

Fig.3.

of the expression changes for the selected genes seen in various AD transcriptional profiles. The expression levels are scale according to $2\frac{(d-c)}{(d+c)}$, where d and c are disease and control values.

Fig.4. A) Graph showing changes in gene expression of neurogenic/proliferation markers across AD studies identified in the GEO human database. B) Graph showing changes in gene expression of neurogenic/proliferation markers in the hippocampus across early AD studies identified in the GEO human database. C) Graph showing changes in gene expression of neurogenic/proliferation markers in the hippocampus across late AD studies identified in the GEO human database. In A, B and C the bars correspond to the number of studies in which gene expression was upregulated (green area) or downregulated (red area) in AD for the individual genes. D) Graph showing the correlation of gene expression changes of neurogenic/proliferation markers with MMSE scores in AD study GSE1297. Genes with a negative correlative Z-score have a negative correlation with MMSE, and therefore, a

positive correlation with AD (red area) whilst genes with a positive correlative Z-score have a positive correlation with MMSE and a negative correlation with AD (green area). GFAP and CRMP1 expression significantly correlated to moderate cognitive impairment (MMSE>15) and *MKI67* significantly correlated to mild cognitive impairment (MMSE>20). E) Graph showing the correlation of gene expression changes of neurogenic/proliferation markers with CDR scores (full range 0-5) in AD study GSE84422. F) Graph showing the correlation of gene expression changes of neurogenic/proliferation markers with CDR scores (range 0-2) in AD study GSE84422. In E and F, genes with a negative correlative Z-score have a negative correlation with CDR, and therefore, AD (red area), and genes with a positive correlative Z-score have a positive correlation with CDR and AD (green area). MMSE = Mini mental state examination. CDR = clinical dementia rating.

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Marker	Full name	Expression
GFAP	Glial fibrillary acidic protein	neural stem cells and astrocytes
VIM	vimentin	expressed during transition from neuroepithelial cell to radial glia and persists during astrocyte development.
Msi-1	Musashi-1	proliferating neural stem cells
GFAPδ	Glial fibrillary acidic protein - delta	pluripotent neural cells
SOX2	(sex determining region Y)-box 2	neural progenitor cells
Nestin	neuroectodermal stem cell marker	intermediate filament protein, implicated in the radial growth of the axon. Can be expressed in glial cells following cerebral ischemia, traumatic brain injury, de-afferentiation of the DG and neurotoxicity.
NeuroD	Neurogenic Differentiation	a differentation factor for neurogenesis. Expressed in later stages of neuronal commitment.
TUC-4 (CRMP-4)	"Turned on after division"	postmitotic neurons at the stage of initial differentiation and is associated with axonal outgrowth. Expressed in the growth cone.
PSA-NCAM	polysialyliated -Neural cell adhesion molecule	Involved in neurite outgrowth and synaptogenesis. Expressed at later stages of neurogenesis including young postmitotic neurons. Important regulator of hippocampal plasticity.
DCX	doublecortin	late mitotic neuronal precursors and early postmitotic neurons.
β-III-tubulin / TuJ1	beta-III-tubulin	immature postmitotic neurons and differentiated neurons and some mitotically active neuronal precursors.
Calretinin	29k Calbindin	immature postmitotic neurons. Mainly expressed during axonal and dendritic targeting.
Calbindin-D28k	Calcium-binding protein D28k	mature dentate granule cells
MAP2c	Microtubule-associated protein 2 (Low molecular weight)	most prominently expressed in the developing brain.
MAP2a,b	Microtubule-associated protein 2 (High molecular weight)	mature neurons, reactive astrocytes
HuC/D	Elav-like proteins	early and late postmitotic mature neurons. involved in the differentiation and/or maintenance of neurons.
NeuN	Feminizing Locus on X-3	early and late postmitotic neurons

Proliferation markers

PCNA	Proliferating cell nuclear antigen	present during G1/S phase of the cell cycle
Ki-67	MK167/cellular marker for proliferation	present during all active cycles of the cell cycle

Alzheimer's disease (AD)

Study	*number of cases, sex, Age (Mean±SD)*	*PMD (Mean ±SD)*	*Disease severity scale (Braak)*	*MMSE (mean)*	*Disease duration (years)*	*Additional measures*	*Neurogenic Marker measured*	*Technique used and brain region studied*	*Antibodies used*	*Results*
(Gillian, et al., 1994)	WB Controls n=1, 71 AD n=3, 73.67	WB Controls 24h AD 22h					NCAM	*WB/ELISA* Frontal cortex Temporal cortex hippocampus	NCAM: Polyclonal antisera (Affinity Ltd., Nottingham, UK) 1:3000 dilution	↔ NCAM in AD (through all brain regions).
	ICC Controls n=unknown, 70 ± 14 AD n=unknown, 80 ± 7	ICC combined PMD of 31 ± 3 h								
(Mikkonen, et al., 1999)	Controls n=10 (5m, 5f) 71.1 ± 12.7 AD n=12 (1m,11f) 82.4 ± 10.9	Controls 24. 0 ± 31.3h AD 4.3 ± 1.7h	Controls 0-I AD VI	Controls N/A AD 3 ± 4.2	Controls 0 y AD 9.9 ± 4.1 y		PSA-NCAM	*IHC* DG CA1	PSA-NCAM: Mouse monoclonal 12E3(gifted from Dr. T. Seki, Japan) 1:800	↑PSA-NCAM in AD in outer molecular layers and inner third of DG. ↑PSA-NCAM in AD in some CA1 subfield sections ↔ PSA-NCAM in GCL
(Jin, et al., 2004)	*WB* Controls n=7 (5m, 2f) 35.4 Early AD n=3 (3m) 76.67 Moderate AD n=3 (3m) 79 Severe AD n=3 (3m) 77.33	*WB* Controls 9.28h Early AD 14h Moderate AD 15.67h Severe AD 14.33h					*WB* DCX PSANCAM NEuroD Tuc-4 Calbindin	*WB* hippocampus	DCX: goat polyclonal (Santa Cruz Biotechnology; 1:200), PSA-NCAM: mouse monoclonal (Chemicon; 1:500), NeuN: mouse monoclonal (Chemicon; 1:250) TUC-4: rabbit polyclonal (Chemicon, 1:10,000), NeuroD: goat polyclonal (Santa Cruz Biotechnology; 1:200) Calbindin: mouse monoclonal (Oncogene Science; 1:10,000)	*WB* ↑DCX, PSA-NCAM, Tuc-4 and NeuroD in AD hippocampus ↔ in calbindin D28K and NeuN.
	IHC Controls n=4 (3m, 1f) 66 AD n=5 (1f, 4 unknown) 74.8	*IHC* Controls 15.5h AD 11h					*IHC* DCX Tuc-4	*IHC* SGZ GCL		*IHC* ↑TUC4 and DCX in GCL in AD. *Shrunken, dead DCX/TUC-4 +ve cells observed in SGZ in AD and aged controls.* *DCX+ve cells observed in CA1 in AD.*
(Ziabreva, et al., 2006)	Controls n=7 (3m, 4f) 79.67±3.93 AD n= 7 (1m,6f) 82.50±4.97	Controls 38±15.39h AD 22.25±11.32 h	Controls 2.17 ±1.47 AD 5.17 ±0.98	Controls ND AD 9.83±8.33	Controls 0 y AD 3.92±2.38 y	CERAD	Msi-1 nestin GFAP	*IHC* SVZ ECL	Msi-1: Rabbit polyclonal (Chemicon, 1:1000) Nestin: Rabbit polyclonal (Chemicon, 1:600)	↑Nestin in SVZ in AD . ↔ in ECL. ↓ in Msi-1 in SVZ in AD. ↔ in ECL. ↔GFAP in SVZ

									GFAP: Rabbit polyclonal (DakoCytomation, 1:4000)	*Inverse correlation of Msi1 immunoreactivity in SVZ with ChAT in temporal cortex.*
(Boekhoorn, et al., 2006)	Controls n=10 (6m, 4f) 67.1± 2.3 AD n=9 (4m, 5f) 66.2± 2	Controls 9.42± 5.55h AD 5.12± 1.06h	Controls 0-II AD V-VI		AD 10.7 y	fixation time Reisberg stage Brain weight APOE cause of death age of onset comorbidities	DCX GFAP Ki67	*IHC* SGZ DG hilus CA Cortex	DCX: Goat polyclonal (Santa Cruz Biotechnnology, 1:200) GFAP: mouse monoclonal clone 6F2 (Monosan B.V, Uden, NL, 1:10) Ki67: MIB-1 mouse monoclonal (DakoCytomation, 1:250)	↑Ki67 in CA1-3 in presenile AD (mainly due to increases in glia-rich and blood vessel-rich areas) *Ki67 immunoreactivity in neurons is limited to DG in presenile AD.* ↑GFAP in DG in presenile AD (astrogliosis) ↔ DCX in presenile AD (SGZ, hilus) *(highly variable immunostaining between subjects)*
(Li, et al., 2008)	Controls n=15 (6m,9f) 83.6 ± 7.4 AD n=14 (7m,7f) 79.4 ± 10.9	Controls 2.6 ± 0.6h AD 2.4 ± 0.6h	Controls I-III AD IV-VI			CERAD, APOE genotype	MAP2a/ b (mature markers) MAP2c (immature markers)	*IHC and insitu hybridization* DG (+ cerebellum)	MAP2a,b and MAP2a,b,c (Sigma-Aldrich, 1:200)	↓ MAP2a,b in AD DG. ↔ MAP2a,b,c (total) in AD DG. *MAP2a mRNA levels decreased in AD DG. MAP2c mRNA levels unchanged in AD DG. No changes in cerebellum.*
(Crews, et al., 2010)	Controls, n=5, 87.0 ± 4.6 early/moderate AD n=7, 86.1 ± 1.7 severe AD n= 7, 80.0 ± 1.9	Controls 9.5 ± 3.5 h early/moderate AD 11.8 ± 2.8h severe AD 8.2 ± 0.8h	Controls 0-I early/moderate AD I-II severe AD VI	Controls 28.5±0.9 early/moderate AD 27.6±1.8 severe AD 5.8±4.2	Controls 0 y early/moderate AD 2.7±2.67 y severe AD 10.2±1.4y	Blessed score, Dementia rating scale, Brain weight, years of education	DCX SOX2	*IHC* SGZ	DCX: goat polyclonal (Santa Cruz Biotechnology). SOX2: mouse monoclonal	↓ DCX and SOX2 in severe AD DG. (*associated with elevated levels of potential neurogenesis regulator BMP6)*
(Perry, et al., 2012)	Controls n=21 (8m, 13f) 80.9±8.5 AD n=20 (7m, 13f) 81.2±7.0	PMDs not given (said to be non-significantly different between groups)	Controls 0-III AD IV-VI				Msi-1 Nestin DCX PSA-NCAM B-tubulin	*IHC* SVZ SGZ GCL	Msi-1: rabbit polyclonal, (Chemicon), Nestin: rabbit polyclonal, (Chemicon), DCX: rabbit polyclonal (Abcam) PSA-NCAM: mouse monoclonal (Dako, Glostru p,) β-III-tubulin: mouse monoclonal (Sigma).	Msi-1 (rabbit polyclonal, Chemicon), nestin (rabbit polyclonal, Chemicon), PSA-NCAM (mouse monoclonal, Dako, Glostru p, Denmark), ChAT (goat polyclonal, Chemicon), doublecortin (rabbit poyclonal, Abcam) and β-III-tubulin (mouse monoclonal, Sigma), respectively.

(Gomez-Nicola, et al., 2014)	Controls for CJD n=10 (5m, 5f) 20-35y range Controls for AD n=9 (5m, 4f) 58-79y range CJD n=10 (5m, 5f) 20-34y AD n=10 (5m, 5f) 58-76y				Ki67 Calretinin SOX2	*IHC* DG	Rabbit anti-Ki67: (Abcam) Rabbit anti-Calretinin: (Millipore) Goat anti-Sox2: (Santa Cruz Biotechnologies)	↑Ki67 and Calretinin in DG of AD and CJD ↔ in SOX2 in DG of AD and CJD
(Ekonomou, et al., 2015)	Braak stage 0-II n=12 (7m, 5f), 80.3 ± 8.4 (dementia n=3) Braak stage III-IV n=11 (3m, 8f) 88.9 ± 8.2 (dementia n=5) Braak stage V-VI n=5 (4m, 1f) 86.8 ± 5.3 (dementia n=5)	PMD given as median (IQR) Braak 0-II: 17.5 (12-28)h Braak III-IV: 25 (7-27)h Braak V-VI: 17.5 (9.5-33)h	n=12, 0-II n=11 , III-IV n=5, V-VI		Nestin DCX PCNA HuC/D GFAP	*IHC* SVZ ECL DG	Nestin: Chemicon, 1:200 DCX: Santa Cruz, 1:200 PCNA: DAKO, 1:1000 HuC/D: Invitrogen, 1:1000 GFAP: DAKO, 1:6000	↔Nestin in DG. ↓HuC/D in DG in Braak V-VI. ↓GFAP in DG in Braak III-IV. ↑GFAP in DG in Braak V-VI. ↑DCX in DG in higher Braak stages. ↔ all markers in SVZ and ECL *(Significant positive correlation between new neurons and activated microglia and a negative correlation between new neurons and astrocytic cell numbers.)*
(Briley, et al., 2016)	Controls n=4 (3f,1m), 74->89y range MCI n=3 (3f), ≥89y. AD n=6 (5f,1m), 67->89y range NDAN n=4 (3f,1m) >89y.	Controls 2-16h range MCI 4-20h range AD 3.3 – 25h range NDAN 4.5-48h range	Controls I-II MCI II-V AD VI NDAN IV-VI	Controls 29-30 MCI 20-25 AD 0-15 NDAN 26-29	SOX2 NeuN	DG	Rabbit anti-SOX2 rabbit (Cell Signaling, 1:200 mouse anti-NeuN (Millipore, 1:1000).	↑total SOX2+ cells in DG in NDAN (n.s. compared to controls, significantly higher than in AD and MCI) ↑SOX2+/NeuN+ in DG in NDAN ↓SOX2+/NeuN+ in DG in AD and MCI ↑SOX2+/NeuN- in DG in NDAN and MCI ↓SOX2+/NeuN- in DG in AD ↔ total NeuN+ in AD, MCI and NDAN ↔ NeuN+/SOX2- in AD, MCI and NDAN

	(Gillian, et al., 1994)	(Mikkonen, et al., 1999)	(Jin, et al., 2004)	(Ziabreva, et al., 2006)	**(Boekhoorn, et al., 2006)**	(Li, et al., 2008)	(Crews, et al., 2010)	(Perry, et al., 2012)	(Gomez-Nicola, et al., 2014)	**(Ekonomou, et al., 2015)**	(Briley, et al., 2016)
GFAP				↔ (SVZ)	↑ **(DG)**					↓ **(DG-Braak III-IV)** ↑ **(DG-Braak V-VI)** ↔ **(SVZ)** ↔ **(ECL)**	
Msi-1				↓ (SVZ) ↔ (ECL)				↓ (SGZ) ↓ (GCL) ↔ (SVZ)			
SOX2							↓ (SGZ)		↔ (DG)		↑ NDAN (DG) ↓AD (DG)
Nestin				↑ (SVZ) ↔ (ECL)				↑ (SGZ) ↑ (GCL) ↑ (SVZ)		↔ **(DG)** ↔ **(SVZ)** ↔ **(ECL)**	
TUC-4/CRMP-4			↑ (GCL by IHC) ↑ (hippocampus by WB)								
PSA-NCAM	↔ (Hippocampus by WB)	↔ (GCL) ↑ (CA1 subfields, outer molecular layers and inner third of DG)	↑ (hippocampus by WB)					↑ (SGZ) ↑ (GCL) ↔ (SVZ)			
DCX			↑ (GCL by IHC) ↑ (hippocampus by WB)		↔ **(SGZ,hilus)**		↓ (SGZ)	↑ (GCL) ↔ (SVZ, SGL)		↑ **(DG-Braak V-VI)** ↔ **(SVZ)** ↔ **(ECL)**	

B-III-Tubulin								↔ (SVZ, SGL, GL)			
Calretinin									↑ (SGZ)		
Calbindin-D28K			↔ (hippocampus by WB)								
MAP2c						↔ (total MAP2a,b,c in DG)					
MAP2a,b						↓ (DG)					
HuC/D										**↓ (DG-Braak V-VI)**	
NeuN			↔ (hippocampus by WB)								↔ NDAN (DG) ↔ AD (DG) [↑ *SOX2+/NeuN+ cells in NDAN (DG)*]

Proliferation markers

PCNA										↔ double PCNA-HuC/D immunolabelling in DG/SVZ	
Ki-67					**↑ (CA1-3)**				↑ (SGZ)		

Study	Are controls defined and age-matched?	Is a disease severity scale given?	Are multiple neurogenic/proliferation markers used?	Is any confounding pathology discussed in controls/patients?
(Gillian, et al., 1994)	IHC- x /WB-✓ control n=1	x	x	x
(Mikkonen, et al., 1999)	✓	✓	x	x
(Jin, et al., 2004)	IHC- ✓/ WB- x	IHC- x /WB-✓	✓	x
(Ziabreva, et al., 2006)	✓	✓	✓	x
(Boekhoorn, et al., 2006)	✓	✓	✓	✓
(Li, et al., 2008)	✓	✓	x	✓
(Crews, et al., 2010)	✓	✓	✓	x
(Perry, et al., 2012)	✓	✓	✓	x
(Gomez-Nicola, et al., 2014)	✓	x	✓	x
(Ekonomou, et al., 2015)	✓	✓	✓	✓
(Briley, et al., 2016)	✓	✓	✓	x